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Frequency of Fra X Syndrome Among Institutionalized Mentally Retarded Males in Poland

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Results of cytogenetic studies, performed in a group of 201 institutionalized mentally retarded males, are presented. At least two cytogenetic methods for eliciting the Xq27.3 fragile site, recommended by the Fourth International Workshop on the Fra X Syndrome were used. A subgroup of 67 out of 201 studied males was also examined using molecular methods. In 6 (2.9%) males fra X syndrome was diagnosed. All cytogenetic positive results were confirmed by molecular analysis. Five patients had full expansion CGG repeats and one had both premutation and full mutation. Postulated frequency of fra X syndrome in Polish population being 0.2–0.4/1,000 males seems to be lower than it could be expected on the basis of previous literature data. © 1996 Wiley-Liss, Inc.

KEY WORDS: mental retardation, fra X syndrome, prevalence, cytogenetics, molecular studies

INTRODUCTION

Based on two large epidemiological studies of unselected mentally retarded males, the prevalence of fra X was estimated as 1/1,226 [Blomquist et al., 1983; Webb et al., 1986]. This prevalence documents the fra X as a second to trisomy 21 cause of mental retardation. Improved techniques for fragile X have benefited the families identified and counselled suggesting that systematic screening for fra X syndrome should be an essential component of genetic service [Turner et al., 1992].

The wide range of fra X frequencies reported in the literature [English et al., 1989; Hagerman et al., 1988; Sutherland, 1985; Turner et al., 1986] and the lack of any data on the prevalence of fra X in our country prompted us to estimate this frequency in Polish population. We also wished to validate the use of different, cytogenetic and molecular techniques for fragile X diagnosis as well as for carrier identification.

MATERIALS AND METHODS

A total of 201 unselected mentally retarded males, including 172 from the institution and 29 from the special school, were studied between 1992 and 1994. All of them came from central Poland (Warsaw region). The age of the studied males ranged from 7 to 80 years. According to medical records 173 of them were diagnosed as severely retarded. All patients were screened for fragile site Xq27.3 using cytogenetic methods. A subgroup of 67 out of 201 studied males was also examined for CGG amplification. All cytogenetic positive results were verified by molecular analysis and proband's relatives were also examined.

Cytogenetics

Cytogenetic studies were performed according to guidelines and recommendations for fragile X studies developed at the Fourth International Workshop on the Fragile X Syndrome and Mental Retardation [Jacky et al., 1991]. Two and in some cases three fra X induction systems were used for whole blood cultures. Lymphocytes were grown in folate thymidine deficient medium TC 199 or in the presence of Methotrexate (5 μg/ml) [Sutherland, 1979] or excess of thymidine (600 μg/ml) for the last 24 hr of culture [Sutherland et al., 1985a]. At least 100 unbanded cells were screened for fragile X chromosome. The presence of Xq27.3 fragile site was always confirmed with GTG banding technique. Constitutive folate sensitive fragile sites such as 1q43, 3p14, 6q26, and 16q23 were also registered (as a control of the work of fragile site induction system). All fragile X studies included a regular constitutional chromosome analysis.

Molecular Studies

Standard methods were applied for DNA extraction from leukocytes [Miller et al., 1988]. Southern blot

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TABLE I. Results of Cytogenetic Studies in Institutionalized Mentally Retarded Males

No. studied	Fra X positive cases (%)	Abnormal karyotype (%)	
		Down syndrome	Other aberrations
201	6 (2.9)	23 (11.4)	4 (1.9)[a]

[a] 46,XY/47,XY,+mar = 2 cases; inv(8)(p11.2q24.3) = 1 case; inv(11)(p15.1q21) = 1 case.

analysis, according to Rousseau et al., [1991] and PCR method, according to Fu et al. [1991], were used to search for abnormal expansion of CGG repeats and hypermethylation of adjacent CpG island.

Prevalence Estimation

The extrapolation of cytogenetic results for prevalence estimation needs several assumptions concerning the selection mechanism of institutionalized individuals. For the purposes of this study the following assumptions based on the data of Wald et al. [1977] have been applied: 1) The prevalence of mental retardation is 2–3%. 2) Males constitute a higher proportion of mentally retarded people than females (60% vs. 40%). 3) The proportion of institutionalization among mentally retarded is 26–35% and changes neither with age nor with other characteristics (e.g., social status, place of residence). The sensitivity analysis accounting for given assumptions was used for estimating the prevalence rate of institutionalized mentally retarded men.

RESULTS

The fra X q27.3 was identified in 2.9% males (Table I). Mean frequency of fragile site expression ranged from 10.7 to 37.9%. In all identified fra X positive cases the presence of methylated full mutation was documented. Five patients had only large expansion of CGG repeats and one had both premutation and full mutation (mosaic). Using pedigree data molecular studies were performed in 9 healthy relatives of the affected males (Table II). In 4 cases carrier status was excluded.

The sensitivity analysis shows that the prevalence rate of institutionalized mentally retarded males in the population equals to no more than 1.2% and no less than 0.6% (Table III). Multiplying these figures by 3% of the fragile X syndrome proportion among institutionalized mentally retarded males allows to estimate the prevalence rate of fragile X syndrome in the Polish population as not exceeding 0.35 per 1,000 men and not being less than 0.17 (Table IV).

COMMENTS

Present results confirm the finding of others, that after the Down syndrome, the fragile X is second largest group of cytogenetically abnormal males in institutions for the mentally handicapped [English et al., 1989; Gustavson et al., 1986]. The frequency of fragile X males found in our studies is within the range of other published data varying from 0 to 11% [English et al., 1989; Hagerman et al., 1988; Sutherland, 1985b; Turner et al., 1986].

At the beginning of our study the diagnosis of fragile X syndrome has been based on the cytogenetic detection of the fragile site Xq27.3. Identification of FMR-1

TABLE II. Results of Molecular Studies in Fragile X Families*

Pedigree number	Family members	Genotype (Southern blots)	CGG number (PCR test)[a]	Methylation of CpG island (Southern blots)	% fra X positive cells
5720	Propositus	fm	LA	met	11
	Mother	p/–	107/30	–/–	0
	Sister	–/–	30/29	–/–	0
5763	Propositus	fm + p	119	met	38
	Brother (affected)	fm	LA	met	32
	Mother	fm/–	30	met/–	0
5901	Propositus	fm	LA	met	18
	Mother	p/–	80/29	–/–	0
	Sister	p/–	29[b]	–/–	0
	Brother 1	–	29	–	ns
	Brother 2	–	29	–	ns
5940	Propositus	fm	LA	met	30
	Mother	p/–	91/22	–/–	0
6279	Propositus	fm	LA	met	27
	Sister	–/–	32/29	–/–	0

* fm = full mutation, p = premutation, LA = lack of amplification, met = methylation of CpG island (in the case of females—methylation of active, normally unmethylated X chromosome), ns = not studied.
[a] Alleles with about 130 or more repeats (full mutations and large premutations) did not amplify.
[b] In this case the premutation was larger than usual (about 200 repeats), therefore it did not amplify in PCR reaction.

TABLE III. Prevalence Rate of Institutionalized Mentally Retarded Men—Sensitivity Analysis

Percentage of institutionalized mentally retarded	Percentage of mentally retarded men in population	
	2.2	3.3
26	0.572%	0.858%
35	0.770%	1.155%

gene [Verkerk et al., 1991] allowed the development of more sensitive and specific molecular diagnostic tests which replaced cytogenetic analysis. The molecular methods introduced to our study allowed not only to verify cytogenetic diagnosis but also to identify carriers of the mutation among proband's relatives. Moreover we were able to show, documented by others [Oostra et al., 1993], superiority of direct DNA analysis of the fragile X mutation especially for carrier detection. Using only cytogenetic methods we were not able to demonstrate carrier status for the premutation in 6 female proband's relatives.

The comparison of our data with the frequency of fragile X syndrome among unselected institutionalized mentally retarded males with results obtained in other studies is difficult. The reason for this is the different proportion of individuals with severe and mild mental retardation and different policy of institutionalization of mentally impaired persons. The estimated interval of prevalence rate of fra X in the Polish population equals to 0.17–0.35 per 1,000 males. In population based studies on males from other countries the point estimators were from 0.4 to 0.8/1,000 and are twice as high as in our studies [Gustavson et al., 1986; Kähkönen et al., 1987; Turner et al., 1986]. However, our prevalence rate corresponds to the prevalence figures presented recently by Turner et al. [1996]. According with their results based on molecular studies the incidence of fra X syndrome is 1:4,000 males and is considerably lower than previously thought.

Our results present the first attempt to estimate the prevalence of fra X syndrome in Polish population. Being conscious of the possible influence of selection bias, we applied a new approach to this process (sensitivity analysis), which directs design of future studies.

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TABLE IV. Prevalence Rate of Fragile X Syndrome*

Percentage of institutionalized mentally retarded	Percentage of mentally retarded men in population	
	2.2	3.3
26	0.017%	0.026%
35	0.023%	0.035%

*Results presented in Table III multiplied by 3%.

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